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None

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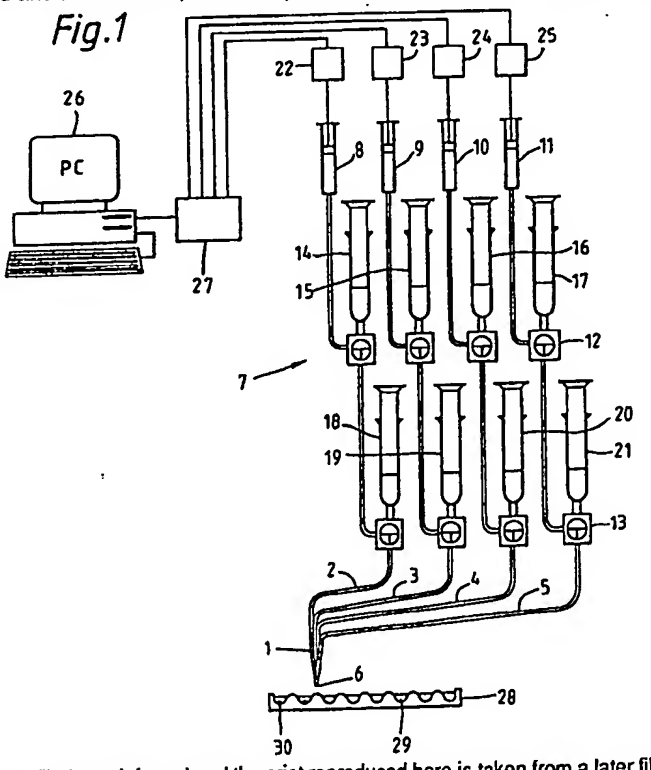
(54) Crystallisation method and apparatus

(57) A method and apparatus for crystallising a material such as a protein is provided. A dispenser (1) constituted by four fluoropolymer tubes (2 to 5) formed into a common tip (6) dispenses a droplet into a container (28) containing an oil (29) immiscible with the droplet. Droplets are formed having different proportions of solvents, precipitants and additives, and stored as a sequence of droplets having a predetermined progression of solution parameters. Mixing of the sample of the material to be crystallised and the solvents, etc takes place only as the droplet is being dispensed from the top (6) of the dispenser (1).

Fig.1



At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

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Fig.1

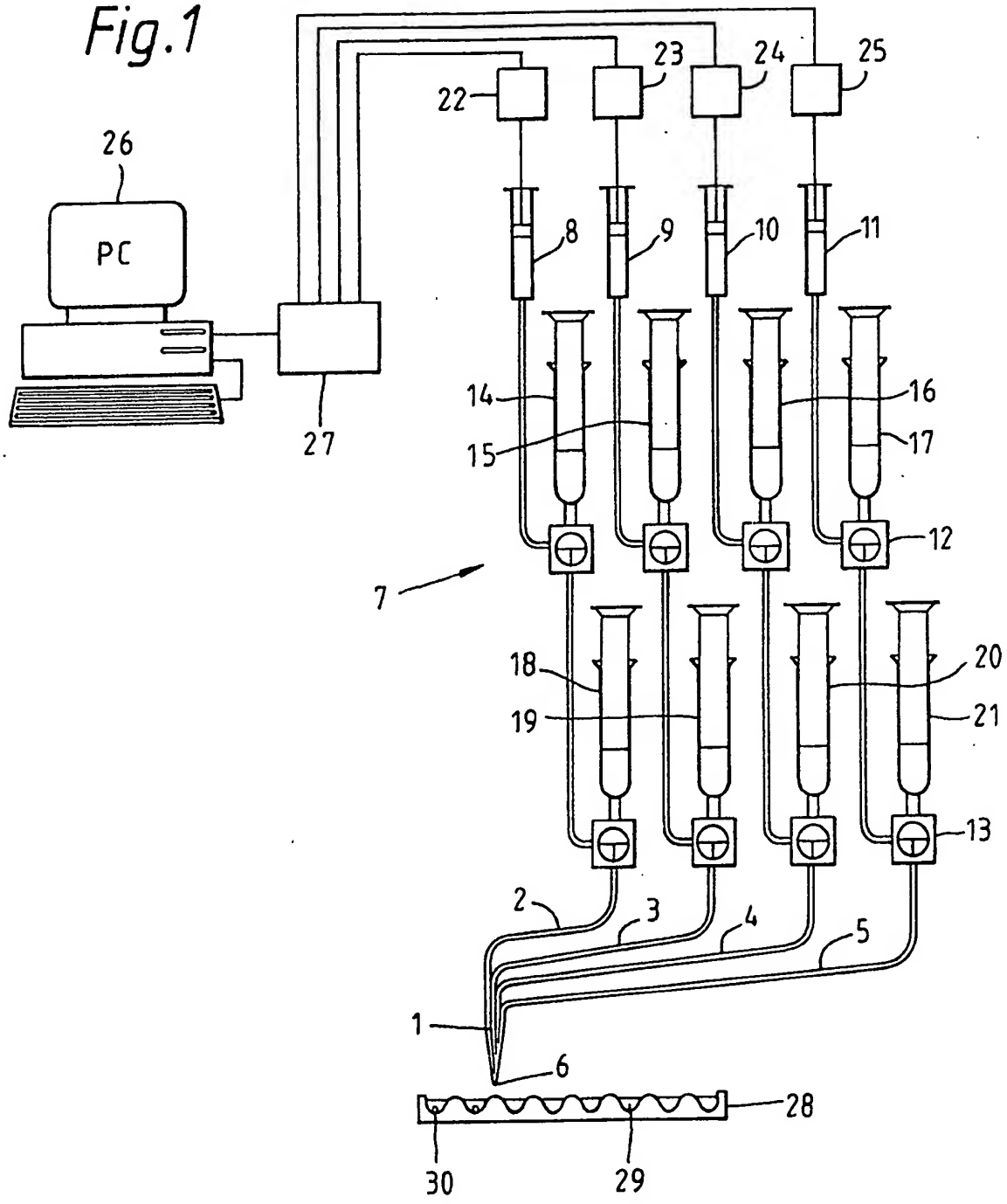
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Fig. 2

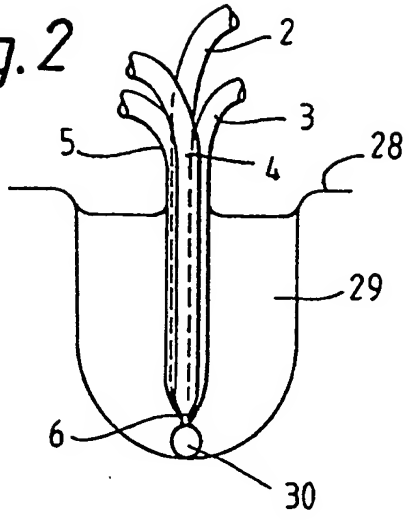


Fig. 3

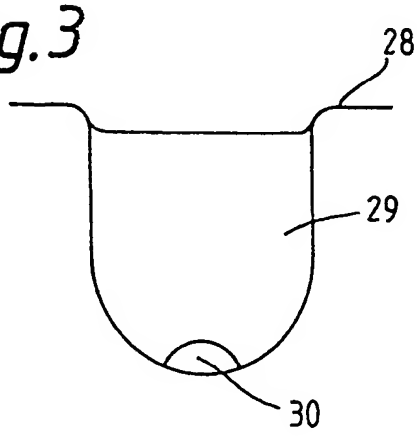
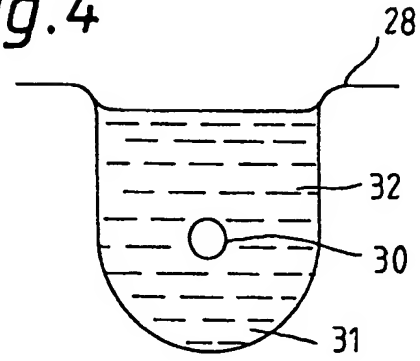


Fig. 4



CRYSTALLISATION OF MATERIALS

This invention relates to a method and apparatus for the crystallisation of materials, particularly materials which are often difficult to crystallise, such as proteins.

The structure of a macromolecular material, such as a protein, can be investigated by X-ray diffraction techniques provided suitable crystals of the material can be obtained. However, many proteins are difficult to crystallise effectively, and automated apparatus for carrying out protein crystallisation experiments has been proposed.

Such automated systems are known to employ the vapour diffusion method of crystallisation, such as the 'hanging drop' method in which a receptacle is part-filled with a buffer solution, a droplet of the buffer solution is transfered to a cover slip, and then a droplet of protein solution is added to the buffer solution on the cover slip. The cover slip is then placed over the receptacle with the droplet of protein solution hanging from the cover slip over the buffer solution in the receptacle. As the buffer solution diffuses from the droplet, protein is encouraged to crystallise.

One such apparatus for carrying out this 'hanging drop' method is described by Cox and Webber in J.Appl Cryst. 20 pages 366-373 (1987). The apparatus of Cox and Webber includes two pipettes, a coarse pipette for filling the receptacles with buffer solution and a finer pipette for manipulating the droplets.

The vapour diffusion method suffers from several disadvantages, including the disadvantage that the precise crystallisation conditions are difficult

to determine. The present invention provides an alternative method of crystallisation, and apparatus suitable for carrying out such crystallisation experiments.

Accordingly there is provided a method of crystallising a material comprising the steps of providing a solution containing a sample of the material to be crystallised; dispensing a droplet of the solution into a container containing a liquid immiscible with the solution; and storing the droplet within the liquid to allow crystallisation of the material to take place.

The invention further resides in a method of crystallising the material comprising the steps of:

1. providing a sample of the material to be crystallised;

2. mixing the sample with one or more liquid materials to form a solution;

3. dispensing a droplet of the solution into a container containing a liquid immiscible with the solution;

4. repeating steps 1. to 3. with different proportions of the one or more liquid materials and/or sample of the material to be crystallised so as to produce a sequence of droplets having a predetermined progression of solution parameters; and

5. storing the droplets within the immiscible liquid to allow crystallisation of the material to take place.

The one or more liquid materials used to form a solution of the sample to be crystallised may be solvent

materials, or additionally solute materials such as precipitants or additives. The term 'solvents' is herein used to include all such liquid materials. Preferably the method includes the step of dispensing the droplet of the solution by means of a dispenser having a tip, the droplet being dispensed from the tip of the dispenser when the tip is immersed in the immiscible liquid. By dispensing and storing the droplet within the immiscible liquid, evaporation of the droplet is minimised. In addition, the droplet is cushioned and buoyed by the immiscible liquid adding to the physical robustness and making the droplet more easily transportable. Preferably the immiscible liquid is an oil such as liquid paraffin oil or silicone oil.

The step of mixing the sample with the one or more solvent materials preferably takes place only as the droplet is being dispensed from the tip of the dispenser. Additionally, two or more solvent materials are provided, the mixing of the two or more solvent materials taking place only as the droplet is being dispensed from the tip of the dispenser. This may conveniently be achieved by employing a dispenser comprising three or more separate liquid channels, drawn out finely at the tip. As mixing of the constituents within the dispenser is avoided, the requirement for repeated flushing of the system between experiments is minimised.

The method conveniently includes the step of dispensing the droplet into a container containing first and second liquids, both immiscible with the solution, the first liquid being of a higher density than the solution and the second liquid being of a lower density than the solution, such that the droplet is suspended in the first and second liquids. This has the advantage of

minimising contact between the droplet and the material of the container.

According to a further aspect of the invention there is provided apparatus for crystallising a material, the apparatus including a dispenser having a tip and being adapted to emit liquid therefrom in the form of droplets, the dispenser comprising three or more discrete liquid channels, each of the channels having a separate supply means for supplying a liquid thereto, each of the supply means having a dosing mechanism for causing a predetermined volume of liquid to be emitted from the tip of the dispenser, the arrangement being such that liquid from the three or more liquid channels mixes to form a combined fluid droplet emerging from the tip of the dispenser.

Conveniently the apparatus further includes one or more receptacles each adapted to receive a droplet emitted by the dispenser, a droplet being received in one of the one or more receptacles under conditions such that crystallisation of the material is made possible. Each of the three or more liquid channels of the dispenser are preferably disposed such that mixing of the liquids from each channel takes place only as the droplet is being dispensed from the tip of the dispenser. Preferably the dosing mechanism is adapted to cause liquid to be dispersed from each of the three or more liquid channels substantially simultaneously, so as to encourage the formation of a homogeneously mixed droplet. Conveniently the dispenser comprises three or more tubes, adjacent one to another, and culminating in a common tip through which liquid from any or all of the tubes can be dispensed. The tip of the dispenser is conveniently formed from a material which is water repellent, such as a fluoropolymer material. This helps to ensure that the volume dispensed from the tip is quantitatively

transferred to the receptacle, the only liquid being carried over on the tip to any subsequent receptacle being the immiscible liquid. In one preferred arrangement the dispenser comprises four discrete liquid channels.

Preferably the apparatus further includes an electronic processing unit adapted to control the dosing mechanism of each of the liquid channels. Conceivably the electronic processing unit is further adapted to control a drive means for providing movement of the dispenser.

According to a further aspect of the invention there is provided a method of crystallising a material comprising the steps of providing a dispenser having three or more discrete liquid channels; supplying a sample of the material to be crystallised in one of the liquid channels; supplying solvent materials in the remaining liquid channels; dispensing an independently predetermined volume of liquid from each of the three or more liquid channels, liquid from each of the channels mixing to form a combined fluid droplet at the tip of the dispenser; transferring the droplet to a receptacle; and storing the receptacle under conditions such that crystallisation of the material is made possible.

The invention further resides in a method of crystallising a material comprising the steps of:

1. providing a dispenser having three or more discrete liquid channels;

2. supplying a sample of the material to be crystallised in one of the liquid channels;

3. supplying solvent materials in the remaining liquid channels;

4. dispensing an independently predetermined volume of liquid from each of the three or more liquid channels, liquid from each of the channels mixing to form a combined fluid droplet at the tip of the dispenser;

5. transferring the droplet to a receptacle;

6. repeating steps 4. and 5. with different combinations of volumes of the liquids from the three or more liquid channels so as to produce a sequence of droplets having a predetermined progression of solution parameters; and

7. storing the droplets under conditions such that crystallisation of the material is made possible.

A further feature of this invention is that it allows convenient alteration of the conditions of crystallisation during the process of crystal growth, which is difficult to achieve with other methods. In particular, after a period of incubation of the droplet when nucleation of crystals may have taken place, the tip may be reinserted into the droplet and further solution added to alter the conditions so that no further nucleation occurs, but existing nuclei continue to grow, thus producing a smaller number of larger crystals. Alternatively, when crystal growth has ceased due to depletion of the crystallising material from the solution of the droplet, its concentration can be increased by the addition of further concentrated material from the tip, stimulating further crystal growth. In these cases, careful mixing of the droplet is carried out, using the same tip.

The various aspects of the invention will now be further described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 is a schematic diagram of apparatus according to the invention;

Figure 2 is an enlarged view of a part of the apparatus of Figure 1, showing the dispensing of a droplet;
Figure 3 is a view corresponding to Figure 2 once the droplet has been dispensed; and

Figure 4 is a view corresponding to Figure 2 showing an alternative embodiment of the invention.

Referring to Figures 1 and 2, the apparatus comprises a dispenser 1 constituted by four fluoropolymer tubes 2 to 5 formed into a common tip 6. The tubes are provided with a dosing mechanism shown generally at 7.

The dosing mechanism comprises four motorised syringes 8 to 11 one being connected to each fluoropolymer tube by means of two sets of 3-way valves 12 and 13. Each set of 3-way valves is provided with a set of ground glass syringes, syringes 14 to 17 being connected to the 3-way valves 12, and syringes 18 to 21 being connected to the 3-way valves 13. The motorised syringes 8 to 11 are driven by means of stepper motors 22 to 25, controlled by a microprocessor 26 connected to the stepper motors by means of an interface unit 27.
Below the dispenser 1 is a microtitre plate 28 comprising a plurality of wells containing liquid paraffin oil 29. One such well is shown in Figure 2.

The operation of the apparatus will be described as used to crystallise a protein. The motorised syringes 8 to 11 are primed with a propellant such as silicone oil, the propellant being introduced by means of the syringes 14 to 17. One fluoropolymer tube 2 contains a sample of the protein to be crystallised. This can either be introduced from the syringe 18, or more usually, if the

protein is in short supply, it can be drawn up into the tube 2 from the tip 6 of the dispenser 1. The remaining tubes 3, 4 and 5 contain appropriate solvent solutions, precipitants and additives, introduced by means of the syringes 19, 20 and 21 respectively.

The dispenser 1 is moved so that the tip 6 thereof is submerged in the oil 29 of one of the wells of the microtitre plate 28. The dispenser may be moved manually, or may alternatively be automatically manoeuvred by means of a robot arm (not shown) under the control of the microprocessor 26. Once the dispenser is in place, the microprocessor 26 actuates stepper motor 22 to drive motorised syringe 8 so as to cause a predetermined volume of protein to be propelled along tube 2. Simultaneously, the microprocessor 26 actuates the other stepper motors 23, 24 and 25 to drive motorised syringes 9, 10 and 11 so as to cause appropriate volumes of the liquids in tubes 3, 4 and 5 to be similarly dispensed. The various volumes of liquids emitted from tubes 2 to 5 mix to form a droplet of protein solution 30 at the tip 6 of the dispenser 1, as shown in Figure 2. As the dispenser 1 is withdrawn from the oil 29, the droplet detaches from the tip 6 and sinks to the bottom of the well as shown in Figure 3.

The dispenser 1 is then moved into a different well of the microtitre plate 28 and the procedure is repeated with the microprocessor controlling the apparatus to dispense a droplet with a different combination of volumes of liquid from the four tubes 2 to 5. In this way a series of droplets can be dispensed in the wells of the microtitre plate 28, the droplets having different protein solution parameters. The precise constitution of each protein solution droplet is recorded by the microprocessor 26.

Once a series of droplets 30 has been dispensed, the microtitre plate 28 is covered and stored at an appopriate temperature to allow crystallisation of the protein to occur. This can require several weeks or even months. The apparatus was used to form well-formed lysozyme crystals at 18°C in sodium citrate buffer at pH 4.7, precipitated by various concentrations of sodium chloride. Two forms of glucose isomerase crystals were grown by the system with ammonium sulphate as the precipitant. Trigonal bi-pyramids grew in the presence of thymol and rectangular crystals in the absence of thymol. Studies on the solubility of erythrina trypsin inhibitor (ETI) were carried out at various pH values. Precipitate was observed for most concentrations of NaCL above pH 4. Crystals were obtained at 4°C in 47 mM sodium acetate buffer at pH 5.20 with 0.05, 0.1, 0.15 and 0.2 M of sodium chloride as the precipitant.

Figure 4 shows an alternative arrangement in which the wells of the microtitre plate 28 contain a mixture of two oils 31 and 32. Oil 31 has a relatively high density whilst oil 32 has a relatively low density. The effect of this is that the droplet 30 of protein solution is suspended between the oils 31 and 32, as opposed to sinking to the bottom of the well. This has the advantage of minimising contact between the droplet 30 and the material of the microtitre plate 28.

Although specifically described with reference to the crystallisation of proteins, it will be appreciated that the method and apparatus of the present invention could be used to encourage the crystallisation of any macromolecular material which is often difficult to crystallise.

Claims

1. A method of crystallising a material comprising the steps of providing a solution containing a sample of the material to be crystallised; dispensing a droplet of the solution into a container containing a liquid immiscible with the solution; and storing the droplet within the liquid to allow crystallisation of the material to take place.

2. A method of crystallising a material comprising the steps of:

   1. providing a sample of the material to be crystallised;

   2. mixing the sample with one or more liquid materials to form a solution;

   3. dispensing a droplet of the solution into a container containing a liquid immiscible with the solution;

   4. repeating steps 1. to 3. with different proportions of the one or more liquid materials and/or sample of the material to be crystallised so as to produce a sequence of droplets having a predetermined progression of solution parameters; and

   5. storing the droplets within the immiscible liquid to allow crystallisation of the material to take place.

3. A method according to Claim 1 or Claim 2, including the step of dispensing the droplet of the solution by means of a dispenser having a tip, the droplet being dispensed from the tip of the dispenser when the tip is immersed in the immiscible liquid.

4. A method according to Claims 1 and 2 wherein the step of mixing the sample with the one or more liquid materials preferably takes place only as the droplet is being dispensed from the tip of the dispenser.

5. A method according to Claim 4 wherein two or more liquid materials are provided, the mixing of the two or more liquid materials taking place only as the droplet is being dispensed from the tip of the dispenser.

6. A method according to any of Claims 1 to 5 wherein the immiscible liquid is oil.

7. A method according to Claim 6 wherein the immiscible liquid is paraffin oil.

8. A method according to Claim 6 wherein the immiscible liquid is silicone oil.

9. A method according to any of Claims 1 to 8 including the step of dispensing the droplet into a container containing first and second liquids, both immiscible with the solution, the first liquid being of a higher density than the solution and the second liquid being of a lower density than the solution, such that the droplet is

suspended between the first and second liquids.

10. A method according to any preceding Claim wherein the material to be crystallised is a macromolecular material.

11. A method according to Claim 10 wherein the material to be crystallised is a protein.

12. Apparatus for crystallising a material, the apparatus including a dispenser having a tip and being adapted to emit liquid therefrom in the form of droplets, the dispenser comprising three or more discrete liquid channels, each of the channels having a separate supply means for supplying a liquid thereto, each of the supply means having a dosing mechanism for causing a predetermined volume of liquid to be emitted from the tip of the dispenser, the arrangement being such that liquid from the three or more liquid channels mixes to form a combined fluid droplet emerging from the tip of the dispenser.

13. Apparatus according to Claim 12 wherein the apparatus further includes one or more receptacles each adapted to receive a droplet emitted by the dispenser, a droplet being received in one of the one or more receptacles under conditions such that crystallisation of the material is made possible.

14. Apparatus according to Claim 12 or Claim 13 wherein each of the three or more liquid channels of the dispenser is

disposed such that mixing of the liquids from each channel takes place only as the droplet is being dispensed from the tip of the dispenser.

15. Apparatus according to Claim 14 wherein the dispenser comprises three or more tubes, adjacent one to another, and culminating in a common tip through which liquid from any or all of the tubes can be dispensed.

16. Apparatus according to any of Claims 12 to 15 wherein the tip of the dispenser is conveniently formed from a material which is water repellent.

17. Apparatus according to Claim 16 wherein the tip of the dispenser is conveniently formed from a fluoropolymer material.

18. Apparatus according to any of Claims 12 to 17 wherein the dispenser comprises four discrete liquid channels.

19. Apparatus according to any of Claims 12 to 18 wherein the apparatus further includes an electronic processing unit adapted to control the dosing mechanism of each of the liquid channels.

20. Apparatus according to Claim 19 wherein the electronic processing unit is further adapted to control a drive means for providing movement of the dispenser.

21. A method of crystallising a material comprising the steps of providing a dispenser having three or more discrete liquid channels; supplying a sample of the material to be crystallised in one of the liquid channels; supplying solvent materials in the remaining liquid channels; dispensing an independently predetermined volume of liquid from each of the three or more liquid channels, liquid from each of the channels mixing to form a combined fluid droplet at the tip of the dispenser; transferring the droplet to a receptacle; and storing the receptacle under conditions such that crystallisation of the material is made possible.

22. A method of crystallising a material comprising the steps of:

    1. providing a dispenser having three or more discrete liquid channels;

    2. supplying a sample of the material to be crystallised in one of the liquid channels;

    3. supplying solvent materials in the remaining liquid channels;

    4. dispensing an independently predetermined volume of liquid from each of the three or more liquid channels, liquid from each of the channels mixing to form a combined fluid droplet at the tip of the dispenser;

5. transferring the droplet to a receptacle;

6. repeating steps 4. and 5. with different combinations of volumes of the liquids from the three or more liquid channels so as to produce a sequence of droplets having a predetermined progression of solution parameters; and

7. storing the droplets under conditions such that crystallisation of the material is made possible.

23. A method according to Claim 21 or Claim 22 wherein the material to be crystallised is a macromolecular material.

24. A method according to Claim 23 wherein the material to be crystallised is a protein.

25. A crystallised material produced by the method of Claims 1 to 11.

26. A crystallised material produced by the apparatus of Claims 12 to 20.

27. A crystallised material produced by the method of Claims 21 to 24.

28. Apparatus substantially as hereinbefore described with reference to the accompanying drawings.

Patents Act 1977
Examiner's report to the Comptroller under ction 17 (The Search Report)

Application number

8928190

Relevant Technical fields

(i) UK Cl (Edition K) B1G: GX; GZ

(ii) Int Cl (Edition 5) B01D; C30B

Search Examiner

B J GARDNER

Databases (see over)

(i) UK Patent Office

(ii) NONE

Date of Search

10 JANUARY 1992

Documents considered relevant following a search in respect of claims 1 TO 28

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
	NONE	

Category	Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

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